Arsenic Removal from Aqueous Media using *Scenedesmus obliquus*: the Promoting Impact of Microalgae-Bacteria Consortium

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Abstract

The present study was conducted to investigate efficacy of unicellular microalgae *Scenedesmus obliquus* either as solo culture or in combination with the bacterium *Shewanella* sp. in arsenic removal from aqueous media. For this purpose, pure culture of the microalgae was fed with arsenic solution and removal rate was monitored. Moreover, impact of pH, temperature and initial biomass was studied. In the second phase, a consortium of *S. obliquus-Shewanella* was prepared and used to investigate the effect of bacterium presence in terms of removal enhancement. The results showed that at pH 7.0, temperature 29°C and 0.8g/l initial biomass the highest rate of arsenic removal achieved. Comparison of pure culture of *S. obliquus* and *S. obliquus-Shewanella* consortium revealed that the latter significantly enhanced removal efficacy.

Keywords:Phycoremediation, *Scenedesmus obliquus, Shewanella* sp., Arsenic removal.

Introduction

The difficulty related to remediation of pollutants in environment appear to be far more than what it was thought a couple of decades ago. Advantages of microorganisms for decomposing various types of pollutants has been recognized since many years ago. For many years, humans have used bioremediation techniques for elimination of harmful residues of urban sewage, underground water and farmland soil (Jafari et al., 2015). Bioremediation is a process by which the dynamism or toxicity of contaminated materials in one place is eliminated or reduced using biological processes. In fact, bioremediation is a process by which management practices is used for the decomposition or removal of both organic and inorganic pollutants from soil, water, wastewater or any polluted area (Chibuike and Obiora, 2014). The main advantage of bioremediation lies in the fact that it is less expensive and more sustainable than other remediation alternatives (Turki et al., 2017), so it is widely used to treat wastes including wastewater, industrial waste and solid waste (Duarte et al., 2019).

All elements are naturally present at various concentrations in the environment, among which toxic and hazardous elements are also present. This means that natural environments on a global scale are partially contaminated

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by toxic elements. One of the most important threats to natural and human ecosystems is the pollution of water by heavy metals and poisonous metals (Megharaj et al., 2011). Among heavy elements, arsenic (As) has been identified as a carcinogen, and its high concentrations in the ecosystem can be a major concern for public health and the environment. World Health Organization (WHO) has set the permissible limit of arsenic in drinking water at 10 μg/l and various literature have reported this limit for soil which is 20 mg/kg (Sultana et al., 2014). The discovery of groundwater contamination with arsenic in different countries, including Argentina, Chile, China, Bangladesh, United States, India, and recently in Pakistan and Iran shows that arsenic pollution is a global problem and all the waters that are consumed should be tested for arsenic contamination (Vala and Sutariya, 2012). Gold and copper mines represent the major source of arsenic contamination in Iran. In addition, application of pesticides, insecticides and other agricultural inputs has led to entry of arsenic into these resources (Khadem et al., 2019).

The major part of biological sequestering of heavy metals is carried out by microalgae in aqueous media. Microalga capability in absorption and metabolism of heavy elements depend on high surface-to-volume ratio, presence of metal-binding groups with high absorption tendency in the cell wall, and the existence of effective systems for absorbing and storing metal in these organisms (Sulaymon et al., 2013). Microalga may bind up to 10% of their biomass as metals (Leon et al., 2008). In addition to the necessary elements for metabolism, microalga can also absorb high amount of heavy toxic elements. Toxic heavy elements often compete with the rare elements that are essential for binding and absorption into the cell. Heavy elements are elements that are not chemically degradable. As a result, they accumulate in the food chain and the environment (Wang and Chen, 2009). The effective strategies are to refine places infected with heavy elements, to sequester them in unavailable bundles, or to enclose or remove them from contaminated sites (Şen et al., 2015). In terrestrial environments, bacteria, fungi and primary producers (plants and algae) play a major role in the biochemical cycle of heavy metals; while microalga represent major agents for sequestering the heavy elements in aquatic environments (Fazal et al., 2018). The smallest microalgae, which have the highest surface-to-volume ratio, are often the most effective creatures for the sequestration of heavy elements. Regarding these characteristics, it is not surprising that a lot of attention is paid to microalgae for the treatment and purification of sediments and contaminated waste (Kumar et al., 2015).

Beside high efficacy of microalgae in bioremediation of wastewater and polluted sites, it has been proved that presence of some bacterial species as a microalgal-bacterial consortium improve remediation capacity (Solimeno and García, 2017; García et al., 2019). In natural aquatic systems, microalgae produce oxygen for available bacteria and serve as CO_2 sinks. Moreover, interactions between microalgae and bacteria also include other aspects such a production of cell exudates and availability of some growth promoting macromolecules which brings a synergy for microalgae–bacteria consortia to enhance pollutants removal from wastewater (Jia and Yuan, 2016). According to the literature claiming increased efficacy of pollutant removal by algae-bacteria symbiosis, the present study was conducted to evaluate arsenic removal from wastewater by consortium of *Scenedesmus obliquus* (microalgae) and *Shewanella* sp. (bacterium). *S. obliquus* is a microalgae with high efficacy in removal of heavy elements which is widely used for wastewater treatment (Xiong et al., 2016; Gupta et al., 2016; Ansari et al., 2019). *Shewanella* represents a bacterium with unique extracellular electron transfer properties (Hau et al., 2008; Brutinel and Gralnick, 2012) that makes it a potentially ideal choice as remediating agent. Particularly, extracellular electron transfer ability of *Shewanella* plays critical role in biotransformation of heavy metals in environment (Chen and Rosen, 2016).

To this end, the present experiment was conducted to evaluate efficacy of microalgae-bacteria in removal of arsenic from a solution. A consortium of *S. obliquus* and *Shewanella* sp. was formed and its potential in removal of arsenic from aqueous solution was measured through Inductively Coupled Plasma (ICP) Spectroscopy. Briefly, the main objective of this research was to verify the potential of microalgae-bacteria in promoting arsenic removal compared to solo culture of microalgae (*S. obliquus*).

Materials and Methods

Microorganisms preparation

S. obliquus endowed by biology department of Isfahan University and *Shewanella* sp. (IBRC-M 4029) was collected from Iranian Biological Resource Center (Tehran). The microalgae was cultured in Bold's Basal Medium (BBM) at 25°C ± 2 under 16-8 h light-dark photoperiod and light intensity of 200 $\mu mol.m^{-2}s^{-1}$. The flask containing *S. obliquus* was placed on a rotator with 120 rpm rotation speed. Cultures in the exponential growth phase were used in all experimental batch cultures. Bacterial inoculum was cultured in tryptic soy broth (TSB) medium and sub cultured twice to achieve sufficient amount of bacterial biomass.

Experiment set-up

The experiment was carried out through two-step procedure. The first, optimization of arsenic removal by pure culture of *S. obliquus*. For this purpose, arsenic uptake was determined by difference between initial concentration and final concentration of arsenic under each treatment (pH, temperature and biomass) as measured by high resolution ICP-OES spectrometer (SPECTRO ARCOS, Germany). Effect of pH was studied by adding 0.1M NaOH or 0.1M HCl to achieve pH values of 5.0 through 9.0. The effects of increased temperature on arsenic adsorption performance measured by the algal cells at temperature between 23.0 to 35.0 ± 2°C. Five concentrations of microalgae 0.2, 0.4, 0.6, 0.8 and 1 g/l were used to investigate the effect of biomass on the rate of absorption. Effect of each treatment (pH, temperature and biomass) was evaluated separately.

The second phase of the experiment included formation of microalgae-bacteria consortium and evaluation of As removal under co-culture according to optimal conditions obtained in the first phase. In this step, a batch culture of 50ml in a 200 ml-Erlenmeyer flask. Three ratios of microalgae-bacteria namely 1:0 (control), 1:5 and 1:10 (based on cell number) were prepared and used for biosorption assays. Arsenic solution was prepared by dissolving Sodium arsenate dibasic heptahydrate (Na2HAsO4.7H2O, Sigma aldrich) in 1 L of deionized water and added to the Erlenmeyer flasks already containing *S. obliquus*- *Shewanella* mixture to give a total concentrations of 400 ppb. The procedure for arsenic removal from the suspension was conducted for 10 days.

Statistical analyses

All treatments were performed in three replicates according to completely randomized design. *Statistical analyses* were conducted using SPSS, v. 16.0.

Results

Biosorption of arsenic by solo culture of S. obliquus

The results of pH effect on biosorption of arsenic is depicted in Figure 1. As seen, by increasing pH from 5 up to 7, an ascending trend for arsenic biosorption was observed (biosorption of 71% at pH 7.0) after which, by increasing pH to 9.0, biosorption was reduced to 65%. Biosorption of As at pH 7.0 was significantly higher than those observed in other pHs ($P<0.05$).

After determining the optimum pH at 7.0, further assays were performed in this pH value. For evaluating the impact of temperature on As removal from aqueous medium, five temperatures viz. 23, 26, 29, 32 and 35°C were applied. The result of this assay is presented in Figure 2. An apparent increase in As removal can be seen from the graph by increasing of medium temperature from 26°C to 29°C at which significant increase in As biosorption was achieved ($P<0.05$). The temperature of 29°C represents a turning point after which, As removal is gently reduced. Five concentrations of microalgae including 0.2, 0.4, 0.6, 0.8 and 1 g/l were used to evaluate the effect of biomass on biosorption of As. As seen in Figure 3, an ascending trend in As absorption was found by

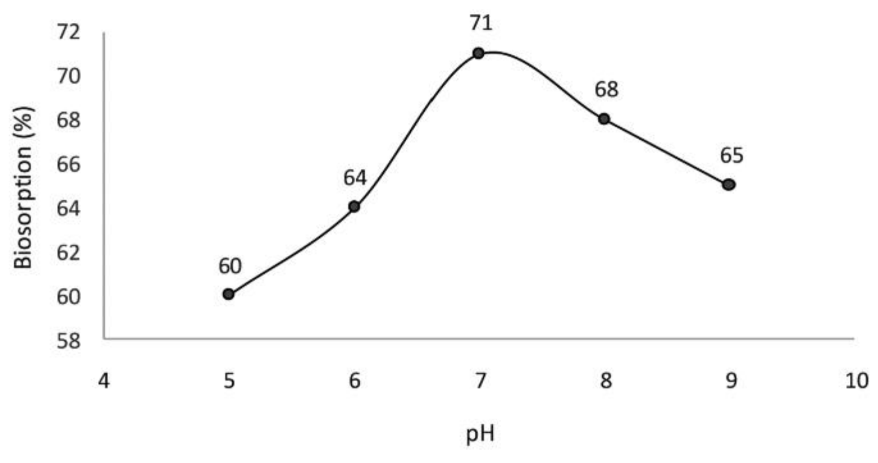

Fig. 1. Biosorption of arsenic by *S. obliquus* as a function of medium pH.

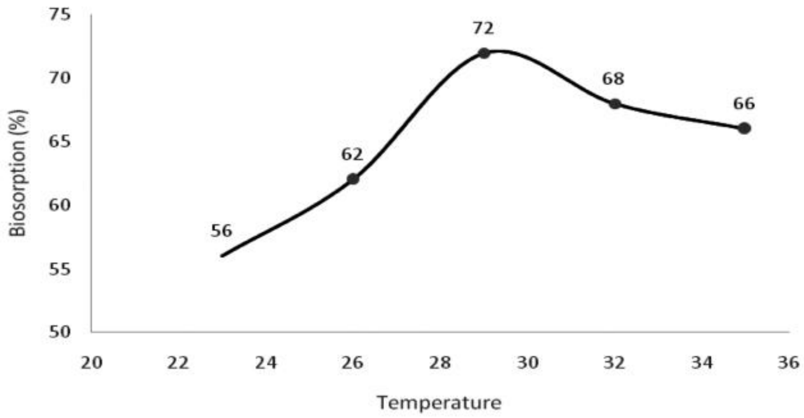

Fig. 2. Effect of various temperatures on biosorption of arsenic by *S. obliquus*.

elevating microalgae biomass from 0.2 up to 0.8 g/l. Further increasing in biomass to 1, biosorption was decreased by 2%; implying the concentration of 0.8 g/l is the best value for As removal from aqueous medium.

As biosorption at initial biomass of 0.8 g/l was significantly higher than concentrations of 0.2, 0.4 and 0.6; but no significant differences were observed for arsenic absorption at concentrations of 0.8 and 1 g/l ($P<0.05$).

Effect of microalgae-bacteria consortium on As removal

According to the findings obtained in solo culture of *S. obliquus*; the optimum condition for removal of arsenic from aqueous medium includes pH 7.0; temperature 29°C; and biomass concentration of 0.8 g/l. This condition was set and used to compare As removal by solo culture of *S. obliquus* and *S. obliquus-Shewanella* consortium. Arsenic removal by pure culture of *S. obliquus* under aforementioned optimal condition is presented in Figure 4. As seen, there is a significant different between *S. obliquus* culture and *obliquus-Shewanella* consortium in terms of As removal ($P<0.05$). However, the difference between two ratios of obliquus -*Shewanella* consortium is not significantly different.

Discussion

The present study aimed evaluating efficacy of the microalgae *S. obliquus* in bio-removal of arsenic from aqueous medium either in solo form or combination with the bacterium *Shewanella*. Microalgae are well known as efficient tools in bioremediation of polluted area and removal of both organic and inorganic pollutants (Jia and Yuan, 2016; Zhang et al., 2018; Hülsen et al., 2018).

However, literature review reveals that most of phycoremediation studies have been focused on removal of organic pollutants such as nitrogen, phosphorus and potassium which are major ingredients of urban wastewater (Cai et al., 2013; Fazal et al., 2018; Rasoul-Amini et al., 2014). Metal elements comprise another category of pollutants with high negative effect on both human life and environment.

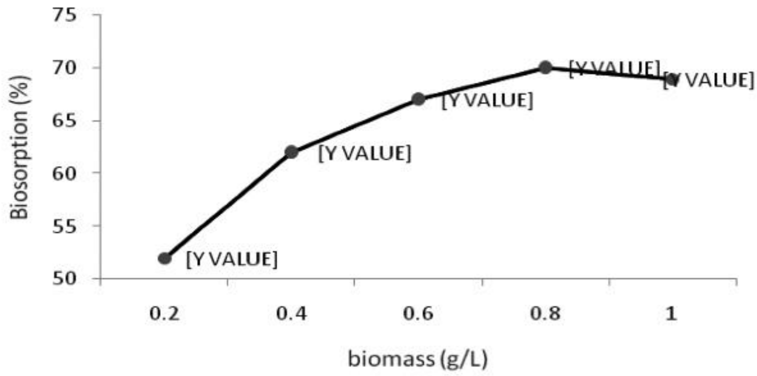

Fig. 3. Effect of various concentrations of *S. obliquus* on biosorption of arsenic.

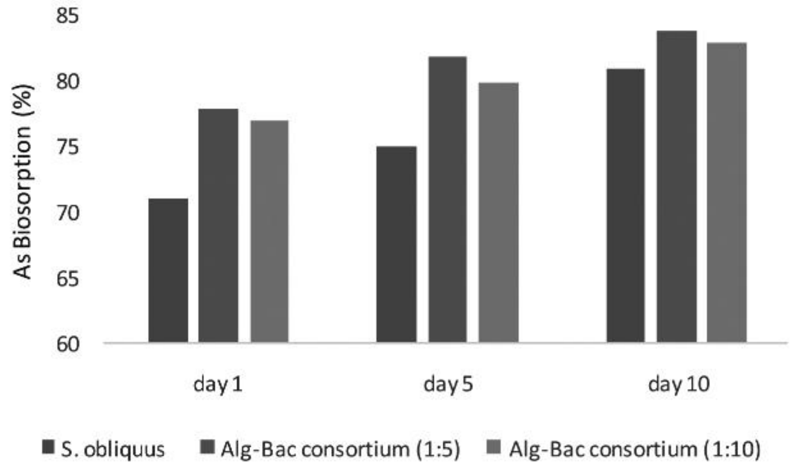

Fig. 4. As removal by *S. obliquus* compared to *S. obliquus-Shewanella* consortium through a 10-days period.

Arsenic represents ubiquitous pollutant with harmful impact on livestock, poultry and human health and is major threat in various mine and industrial sites. The discovery of groundwater contamination with arsenic in different countries shows importance of arsenic pollution as a global problem (Vala and Sutariya, 2012). To this end, removal of arsenic from aqueous media can be a remediation action with great importance. The result obtained in this study confirmed the efficacy of *S. obliquus* in bioremediation of arsenic. This finding is in line with other studies implying ability of *S. obliquus* in removal of cadmium (Monteiro et al., 2009), phosphorus and nitrogen (Martınez et al., 2000; Ji et al., 2013), carbamazepine (Xiong et al., 2016), zinc and copper (Zhou et al., 2012), as well as others. In general, the results of the present study and other researches denotes high capability of *S. obliquus* as a major tool for bioremediation of polluted areas. We investigated effect of pH on improvement of arsenic removal by microalgae culture. The results indicated that pH 7.0 is accompanied with the highest As removal from the solution. pH plays an important role in many cellular processes, which include energy metabolism, structure and function of organelles, enzymes, and proteins (Jia, and Yuan, 2016). pH assay in this experiment shows that by increasing pH to higher values than 7, a sharp decrease in arsenic removal occurs. We argue that pH values upper and lower than 7 interferes with normal growth and biological functions of *S. obliquus* which in turns negatively affects As removal. This increase in arsenic at pH of 7 may be attributed to optimal growth of *S. obliquus* which is observed at neutral pH (Ansari et al., 2019). This argument stems from a large body of studies showing that metal removal by microalgae is an active process in which various biological pathways are involved; so environmental factors such as pH have significant impact on this process (Perales-Vela et al., 2006; Sarı et al., 2011; Turki et al., 2017).

Regarding temperature effect, an increase in As removal was found by increasing medium temperature from 26ºC to 29ºC, after which removal rate was decreased. Every microalgal species thrive in an optimum temperature which provide a suitable condition for its growth and proliferation. Similar to pH impact, temperature affects activities of enzymes and other biological components involved in bioremediation process. Our result obtained on the influence of temperature on As bioremediation is line with the results reported by other authors accentuating the effect of temperature on metal removal by microalgae (Monteiro et al., 2009; Sibi, 2014; Sulaymon et al., 2013). Initial biomass concentration was also found to have significant effect on As removal by *S. obliquus*. The results indicated that initial concentration of 0.8 g/l has the best performance in removal of the metal from aqueous medium. This shows that 0.8 g/l provide sufficient biomass for efficient As removal and concentrations above this value has no significant effect on bioremediation rate. This finding is consistent with Sibi (2014) reported biomass concentrations higher than 0.8 g/l has no significant effect on As removal by *S. obliquus* and *Chlorella vulgaris*.

Removal kinetics was investigated through 10-days period. The highest As removal of 67% from the aqueous medium was observed in first day. The accumulation of heavy metals in algae involves an initial rapid uptake followed by slower uptake. As mentioned frequently by other authors, the large body of metal mass is quickly absorbed in early hours of bioremediation and then a slow trend is observed (Zhou et al., 2012; Sibi, 2014; Kumar et al., 2015; Gupta et al., 2016; Duarte et al., 2019). This may be due to quick saturation of binding sites on microalgae cell surface by metal ions or depleting available enzymes for active removal of pollutants (Leon et al., 2008).

An important part of this research was evaluate the impact of microalgae-bacteria consortium on promoting As removal from the solution. The results obtained in this research regarding the effect of *S. obliquus-Shewanella* consortium on arsenic removal through a 10-day period showed that this approach was fairly successful in improving bioremediation rate. As mentioned in previous section, the consortium (both in 1:5 and 1:10 algae-bacteria ratios) significantly had better performance that pure culture of *S. obliquus* in in absorption. This finding reconfirmed previous reports on usefulness of microalgae-bacteria consortia in promoting pollutants removal and wastewater treatment (Ma et al., 2014; Li et al., 2015; Solimeno and García, 2017). Microalgae-bacteria systems have been proposed as an efficient approach for wastewater treatment and pollutant removal since early 1970 (Humenik and Hana, 1971; McGriff and McKinney, 1972) and there is a growing number of studies examining consortia of different microalgae and bacteria species for their ability to uptake the pollutant from contaminated sites (Zhang et al., 2018). Overall, the results obtained in the present study show that combination of *S. obliquus* and *Shewanella* sp. in consortium form is an efficient, easy to handle and cost-effective approach for removal of arsenic residues in aqueous media. Since arsenic ions released from mines and industrial sites usually enters water resources, the method proposed in the present study may have considerable practical implications. Determining the best ratio of *S. obliquus- Shewanella* consortium for removal arsenic and other metal pollutants under field condition may be the subject of our future research.

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